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ANTICARIOGENIC AGENT [Ushokuyouzai]

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(54) Anticariogenic Agent

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Specification

[Title of the Invention] Anticariogenic Agent

[What is Claimed is]

1. An anticariogenic agent characterized in that it comprises the extract(s) and/or the active component(s) of Magnolia officinalis, Coptis japonica, Rhus javanica Linn, Geranium thunbergii, Gingko biloba leaves, Bletilla striata, Phellodendron bark, Alpinia officiarum, Pulsatilla chinensis, Houttuynia cordata, Prunella vulgaris, Salvia miltiorrhiza, Rheum palmatum, Boswellia carterii, Anemarrhena asphodeloides, Sinomenium acutum, Scutellaria baicalensis, Sasa veitchii, Hydnocarpus anthelmintica Pierre, Lithospermum erythrorhizon, Veronicastrum sibiricum, Paeonia lactiflora, Asarum sieboldii, Cordyceps sinensis, Nandina domestica leaves, Chrysanthemum lavandulifolium, Aurantii Nobilis Pericarpium, Sasa veitchii leaves, Magnolia kobus, Lonicera japonica flowers, Aucklandia lappa Dcne., Picrorhiza kurrooa, Syzygium aramaticum, Cinnamomum Cassia (cinnamon bark), Paeonia suffruticosa bark, Angelica sinensis diels, Portulaca oleracea, Artemisia Montana Pampan, Arctoostaphylos uva-ursi, Artemisia capillaris, Polyporus umbellatus, Poria cocos, and/or Evosia rutaesarpa.

2. An anti-cariogenic agent in accordance with Claim 1, characterized in that its active components are magnolol, honokilol, and/or beriberine.

[Detailed Explanation of the Invention]

The present invention relates to an anticariogenic agent or an oral agent that prevents dental caries and inhibits the progress of dental caries.

Dental caries is generally called cavity and is a disease that causes the teeth to be progressively decayed without limitation. Further, it has a high incidence ratio and is a serious problem in terms of today's public health.

According to the results of recent research, dental caries is caused due to the change of sucrose contained in foods that is caused by the function of a certain oral streptococcus and the formation of insoluble adhesive glucan (polysaccharides comprising D-glucose). In other words, bacteria attach to the teeth and grow due to the glucans produced in the above-described manner to form dental plaque, which is harborage for bacteria. This is the first stage of dental caries. Subsequently, the bacteria progress dental caries using the dental plaque as a base by degrading the tooth tissues by the acids produced through saccharide fermentation.

In this manner, because dental caries is infectious, the oral streptococcus, which is the cause of dental caries, must be destroyed to prevent dental caries or inhibit the progress of dental caries.

Known carious bacteria include Streptococcus mutans, S. sanguis, and S. mitis. Of these, S. mutans is known to have the strongest cariogenicity. In other words, as its salient characteristic, it is known to have an adhesive property on the tooth surface (which requires sucrose), bacteria agglomerate reaction (which forms dextran), and the ability of producing lactic acid due to the fermentation of sorbitol and mannitol. These characteristics are strongly associated with the cause of dental caries.

In order to prevent dental caries, these oral bacteria often have been attempted to be removed. For example, antibiotic substances such as penicillin and erythromycin, cell wall dissolving enzymes, and bactericides such as chlorhexidine have been somewhat available for practical use. However, they destroy the natural balance of bacteria and cause other side effects by disturbing oral and intestinal bacterial flora. Particularly, antibiotic substances develop significant side effects. Thus, any of the substances listed above have not been widely used. In conclusion, there is not an outstanding method at this point to prevent dental caries, and there is no better prevention method than physical cleaning methods.

The inventors considered the above-described points and conducted various investigations aimed at preventing dental caries and inhibiting the progress using Japanese and traditional Chinese medicines. As a result, they discovered that some Japanese and traditional medicines had a superior bactericidal effect against cariogenic bacteria and found the active components. The present invention is based on this discovery. The process of the investigation and the details of the present invention as the result of the investigation are described in the following.

In the first stage, the inventors selected many Japanese and traditional Chinese medicines that have been conventionally known to have any bactericidal effects. The methanol, 50% methanol, and aqueous extracts of the selected Japanese and traditional Chinese medicines were evaluated for their sensibility against S. mutans bacteria by the paper disk method. Types C and D of the 7 blood serum types of the bacteria, which are the most common in Japanese people, were used as targeted bacterial strains. Further, the evaluation was performed using 5 different extract concentrations from (+) to (+++++) according to the evaluation criteria, (-) when the diameter of inhibition circle was smaller than 9 mm and (+) when it was larger than 9 mm as compared with the 8 mm diameter of paper disk. Table 1 shows the crude drugs with superior results. The upper and lower columns of each crude drug shown in Table 1 are the data of types c and d respectively.

Table 1

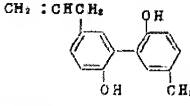
	Methanol extract	50% Methanol extract	Aqueous extract
Magnolia officinalis	++++	+++++	+++
	+++++	+++++	++
Magnolia obovata	++++	++++	+
	+++	++++	+
Coptis rhizome	+++++	+++	+++
	+++	+++	+++
Rhus javanica Linn	++++	+++	++
	+++	++	-
Geranium thunbergii	++++	±	+
	++	+	+
Gingko biloba leaves	++++	+	-
	++	-	-
Bletilla striata	+++	-	-
	+++	-	-
Phellodendron bark	++	++	++
	+++	++	++
Alpinia officiarum	+++	++	-
	+++	-	-
Pulsatilla chinensis root	++	-	-
	++	-	-
Houttuynia cordata herb	+++	+++	-
	+++	++	-
Prunella vulgaris	+++	±	-
	++	+	-
Salvia miltiorrhiza	++	±	-
	++	-	-
Rheum palmatum	++	+	++
	+	+	-
Boswellia carterii	++	-	-
	-	-	-
Anemarrhena asphodeloides	+	++	-
	+	+	-
Sinomenium acutum	+	+	-
	+	-	-
Scutellaria baicalensis	+	-	-
	+++	-	-
Sasa veitchii	±	±	+++
	±	-	++
Hydnocarpus anthelmintica Pierre	-	+	-
	+	++	-
Lithospermum erythrorhizon	-	-	+
	-	-	+
Veronicastrum sibiricum[1]	+	±	-
	+	-	-

[1] Uncertain. Character only faintly legible in the original.—Tr.

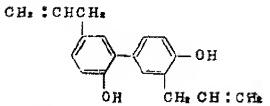
Paeonia lactiflora and Evosia rutaesarpa mentioned in the claim of the present invention also showed superior effects, particularly against type D bacterial strains, but they are omitted to avoid confusion.

Of the tested crude drugs, as shown in the table above, particularly, Magnolia officinalis and Magnolia obovata showed a superior effect. Further, in this experiment, the methanol extracts produced superior results as compared with other extracts. Considering the above-described results, Magnolia officinalis was further investigated.

First, the ethanol extract of Magnolia officinalis was roughly fractionated according to a conventional method. Each fraction was then investigated by the paper disk method. As a result, a bactericidal activity against cariogenic bacteria was developed in the acidic fraction. Further, Magnolia officinalis is commonly known to contain phenol compounds such as magnolol (I) and honokiol (II) (see Yakugaku Zasshi 50, 183, 1930 and 93, 422, 1973), and these two substances were further investigated. As a result, the inventors discovered that both (I) and (II) showed bactericide effects against all of the 7 blood serum types of S. mutans.



Magnolol (I)



Honokiol (II)

Subsequently, the extracts of Magnolia officinalis prepared using other solvents and the extracts of Magnolia obovata were investigated for the bactericidal effects against S. mutans bacteria. Additionally, berberine, which is the antibacterial component contained in Coptis japonica and erythromycin, which is an antibiotic substance known to have a bactericidal effect, were investigated for the bactericidal effects as comparisons.

In other words, the methanol, ethanol, and aqueous extracts of Magnolia obovata; the methanol and aqueous extracts of Magnolia officinalis and Coptis japonica; magnolol, honokiol, berberine and erythromycin were selected and investigated for their antibacterial effects against the 7 blood serum types (types a-g) of S. mutans by the paper disk method. Further, the antibacterial activity was determined by the maximum diameter of inhibition circle using 1.2 mg of each extract and 0.06 mg of each compound. The results are shown in Table 2.

Table 2

		Concentration (mg/disk)	Maximum diameter of inhibition circle (mm)						
			Type a	Type b	Type c	Type d	Type e	Type f	Type g
Magnolia obovata	Methanol extract	1.2	13.4	14.4	14.0	11.7	15.6	16.0	12.7
	Ethanol extract	1.2	15.2	15.3	16.5	13.5	16.1	17.1	13.9
	Aqueous extract	1.2	9.2	-	9.1	8.6	10.1	9.2	9.0
Magnolia officinalis	Methanol extract	1.2	17.8	15.5	18.5	15.8	18.8	21.3	17.4
	Aqueous extract	1.2	12.8	10.0	13.3	10.2	14.3	14.7	13.4
Coptis japonica	Methanol extract	1.2	16.7	16.3	16.5	17.5	17.9	20.6	16.8
	Aqueous extract	1.2	14.1	11.8	12.7	11.8	14.8	14.2	13.3
Magnolol		0.06	18.1	16.0	16.5	17.5	17.9	20.6	16.8
Honokiol		0.06	18.5	15.1	20.4	16.4	20.0	20.9	17.9
Berberine		0.06	10.1	10.9	9.9	9.6	9.6	9.8	10.5
Erythromycin		0.06	36.3	37.4	36.5	39.2	40.5	36.4	37.4

The results shown in Tables 1 and 2 clearly show that the extracts of Magnolia officinalis (including Magnolia obovata, Magnolia officinalis, and others), Coptis japonica, Rhus javanica Linn, Geranium thunbergii, Gingko biloba leaves, Bletilla striata, Phellodendron bark,

Alpinia officiarum, Pulsatilla chinensis, Houttuynia cordata, Prunella vulgaris, Salvia miltiorrhiza, Rheum palmatum, Boswellia carterii, Anemarrhena asphodeloides, Sinomenium acutum, Scutellaria baicalensis, Sasa veitchii, Hydnocarpus anthelmintica Pierre, Lithospermum erythrorhizon, Veronicastrum sibiricum, Paeonia lactiflora, Asarum sieboldii, Cordyceps sinensis, Nandina domestica leaves, Chrysanthemum lavandulifolium, Aurantii Nobilis Pericarpium, Sasa veitchii leaves, Magnolia kobus, Lonicera japonica flowers, Aucklandia lappa Dene, Picrorhiza kurrooa, Syzygium aramaticum, Cinnamomum Cassia, Paeonia suffruticosa bark, Angelica sinensis diels, Portulaca oleracea, Artemisia Montana Pampan, Arctoostaphylos uva-ursi, Artemisia capillaris, Polyporus umbellatus, Poria cocos, and/or Evosia rutaesarpa, and their components such as magnolol, honokilol, and berberine greatly inhibit the growth of S. mutans, which causes dental caries. Therefore, they are extremely useful as anticariogenic agents or oral agents that inhibit the development and progress of dental caries.

Further, although their effect is somewhat weaker than the commonly known erythromycin, the inventive anticariogenic agent is either a crude drug preparation or a substance derived from a crude drug, and therefore, it causes little problematic side effects and significantly reduces the concerns in terms of clinical applications, whereas it is difficult to use the conventional erythromycin repeatedly for an extended period due to the side effects (such as the disturbance of the natural balance of bacteria in the body and the appearance of resistant bacteria) because erythromycin is an antibiotic substance. Particularly, because of its characteristics, an anticariogenic agent is often administered repeatedly or intermittently for an extended period. Therefore, the inventive anticariogenic agent with a low risk of side effects is expected to be extremely useful for practical use. Particularly, Japanese and traditional Chinese medicines such as Magnolia officinalis and Coptis japonica have been long been practically used in practice as internal medicines in the past. Thus, when they are used as crude drug extracts, they are expected to have no side effect.

Further, the inventive anticariogenic agent shows a prompt bactericidal effect against cariogenic bacteria at a low concentration. This is another excellent characteristic of the present invention. This characteristic is explained in the following.

For example, the minimum concentration of berberine to inhibit the growth of S. mutans was evaluated by the bouillon medium dilution method and found to be 67 μg/ml. Further, magnolol and honokiol were found to inhibit the growth of S. mutans bacteria at an extremely low concentration of 7 μg/ml.

Moreover, the relationship between function time and antibacterial effects was investigated by allowing magnolol and honokiol to function against S. mutans bacteria (type c bacterial serum) at a concentration of 70 μg/ml. As a result, the antibacterial effects of both compounds were found to be bactericidal. The compounds were then found to quickly develop the bactericidal effect by a 2 minute contact time and completely kill the bacteria within a 10 minute contact time.

The above-described fact wherein the inventive anti-cariogenic agent develops a significant effect at a low concentration in a short time proves that the inventive product is extremely useful in terms of clinical application.

Further, several crude drugs, extracts, and compounds used in the present invention are already known to have an antibacterial effect.

However, they have been reported to have an antibacterial effect only against pathogens such as colon bacillus, dysentery bacillus, tubercle bacillus, and Staphylococcus aureus but not against the cariogenic S. mutans, as seen in the present invention. Further, the S. mutans bacteria do not belong to any of the categories of Streptococcus by Lancefield and thus are unique bacteria. Therefore, the present invention is a novel, useful, and progressive invention that could not be predicted or suspected from common knowledge.

The inventive anticariogenic agent may be used individually or in a combination of two or more. For example, the ethanol or methanol extract of Magnolia officinalis may be used

individually or together with Coptis japonica. If necessary, magnolol and/or honokiol may be added. Of course, the pure products of magnolol, honokiol, and berberine can be used individually or in a combination. These are of course included in the present invention.

The inventive anticariogenic agent may be directly applied to the oral cavity as it is or mixed with other oral agents such as toothpaste before use. If necessary, it can be in other appropriate forms such as troches and sublingual tablets.

The inventive anticariogenic agent can be used in an appropriate amount according to the results shown in Tables 1 and 2. However, it is preferably used in a slightly larger amount considering the loss of the agent during the application (for example, when it is blended with a toothpaste, a considerable amount is lost by rinsing the mouth). Further, as mentioned above, because the inventive anticariogenic agent hardly develops side effects, it is not expected to have an adverse influence caused by the administration of an excess amount and thus is used without concerns.

Embodiments of the present invention are described as examples in the following. They are only examples used to describe the present invention, and the present invention is not limited to these embodiments.

Embodiment 1

Magnolia obovata was ground and cold-steeped in ethanol. The ethanol extract obtained was separated into acidic, neutral, and alkali fractions according to a conventional method. The acidic fraction was then taken and blended with a commercially available toothpaste to obtain a product.

Embodiment 2

The acidic fraction of the above-described embodiment was processed by column chromatography to isolate magnolol and honokiol. They were dissolved in a small amount of alcohol. Then, a gargle was obtained by adding water and solubilizing agents.

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